13 **Yoneda M**, Fujita T, Yamada Y, Yamada K, Fujii A, Inagaki T, Nakagawa H, Shimada A, Kishikawa M, Nagaya M, Azuma T, Kuriyama M, Wakamatsu N. Late infantile Hirschsprung disease-mental retardation syndrome with a 3-bp deletion in ZFHX1B. *Neurology* 2002;**59**:1637-40.
14 **Garavelli L**, Donadio A, Zanacca C, Banchini G, Della Giustina E, Bertani G, Albertini G, Del Rossi C, Zweier C, Rauch A, Zollino M, Neri G. Hirschsprung disease, mental retardation, characteristic facial features, and mutation in the gene ZFHX1B (SIP1): confirmation of the Mowat-Wilson syndrome. *Am J Med Genet* 2003;**116A**:385-8.
15 **Kääriäinen H**, Wallgren-Pettersson C, Clarke A, Pihko H, Taskinen H, Rintala R. Hirschsprung disease, mental retardation and dysmorphic facial features in five unrelated children. *Clin Dysmorphol* 2001;**10**:157-63.
16 **Nagaya M**, Kato J, Niimi N, Tanaka S, Wakamatsu N. Clinical features of a form of Hirschsprung's disease caused by a novel genetic abnormality. *J Pediatr Surg* 2002;**37**:1117-22.
17 **Hunter AGW**. Brain. In: Stevenson RE, Hall JG, Goodman RM, eds. *Human malformations and related anomalies*. Vol II. Oxford: Oxford University Press, 1993:73.

Molecular study of three cases of odontohypophosphatasia resulting from heterozygosity for mutations in the tissue non-specific alkaline phosphatase gene

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Hypophosphatasia is an inherited disorder characterised by defective bone and tooth mineralisation and deficiency of serum and bone alkaline phosphatase activity. The bone symptoms are highly variable in their clinical expression and range from stillbirths without mineralised bone to pathological fractures developing only late in adulthood.[1] Odontohypophosphatasia is characterised by premature exfoliation of fully rooted primary teeth and/or severe dental caries, often not associated with abnormalities of the skeletal system.[2 3] The anterior deciduous teeth are more likely to be affected and the most frequently lost are the incisors.[4] Dental *x* rays show reduced alveolar bone and enlarged pulp chambers and root canals.[2 4] Although the only clinical feature is dental disease, biochemical findings are generally indistinguishable from those in patients with mild forms of hypophosphatasia (adult and childhood). While perinatal hypophosphatasia and infantile hypophosphatasia are transmitted as an autosomal recessive trait, both autosomal recessive and autosomal dominant transmission may be found in childhood, adult, and odontohypophosphatasia.[3 5–9] The distinction between recessive and dominant transmission may be difficult to determine conclusively by using familial analysis because expression of the disease is very variable, with parents of even severely affected children showing no or extremely mild symptoms of the disease.[9 10]

The tissue non-specific alkaline phosphatase (TNSALP) is a phosphomonoesterase anchored at its carboxyl terminus to the plasma membrane by a phosphatidylinositol-glycan moiety.[11] The enzyme cleaves extracellular substrates pyridoxal-5'-phosphate (PLP), phosphoethanolamine (PEA), and inorganic pyrophosphates (PPi). Its exact function in bone and dental mineralisation is still unclear but probably involves hydrolysis of Ppi[12] and perhaps mammalian specific activities such as collagen and calcium binding.[13]

The *TNSALP* gene is localised on chromosome 1p36.1[14] and consists of 12 exons distributed over 50 kb.[15] More than 127 distinct mutations have been described in the *TNSALP* gene,[16–33] in a relatively small number of North American, Japanese, and European patients,[34] indicating a very strong allelic heterogeneity in the disease. Most of them (82%) were missense mutations. This variety of mutations results in variable clinical expression even among the severe or moderate types. We report here the study of *TNSALP* gene mutations in three patients affected by odontohypophosphatasia and provide evidence that heterozygosity may produce clinical signs and symptoms that appear to be very variable in expression.

Key points

- Hypophosphatasia is an inherited disorder characterised by defective bone mineralisation and deficiency of tissue non-specific alkaline phosphatase (TNSALP) activity. We report here the molecular study of three cases of odontohypophosphatasia where the disease was the result of heterozygosity for *TNSALP* gene mutations.
- Three mutations were found, 323C>T (P91L), 346G>A (A99T), and 1240C>A (L397M). The mutation P91L has not been previously described and site directed mutagenesis experiments showed that it corresponded to a severe allele.
- In one family, the proband's mother carried the mutation P91L and was affected. In the two other families, dominant transmission was more difficult to determine, owing to variable expression of the disease in carriers, ranging from hypophosphatasaemia only to periodontal disease associated with multiple fractures.
- We show here that the convergence of clinical, biochemical, and molecular results may help to affirm the dominant effect of *TNSALP* mutations. Analysis of a 3D model of *TNSALP* indicated that residues affected by these mutations were located near the active site or in the mammalian specific crown domain, corroborating the functional effect of these mutations. This is consistent with the dominant effect of these mutations and the allosteric properties of the enzyme.

MATERIAL AND METHODS

Patients

Patient 1

The proband was a 9 year old boy affected by Down syndrome and odontohypophosphatasia. Loss of seven deciduous teeth,

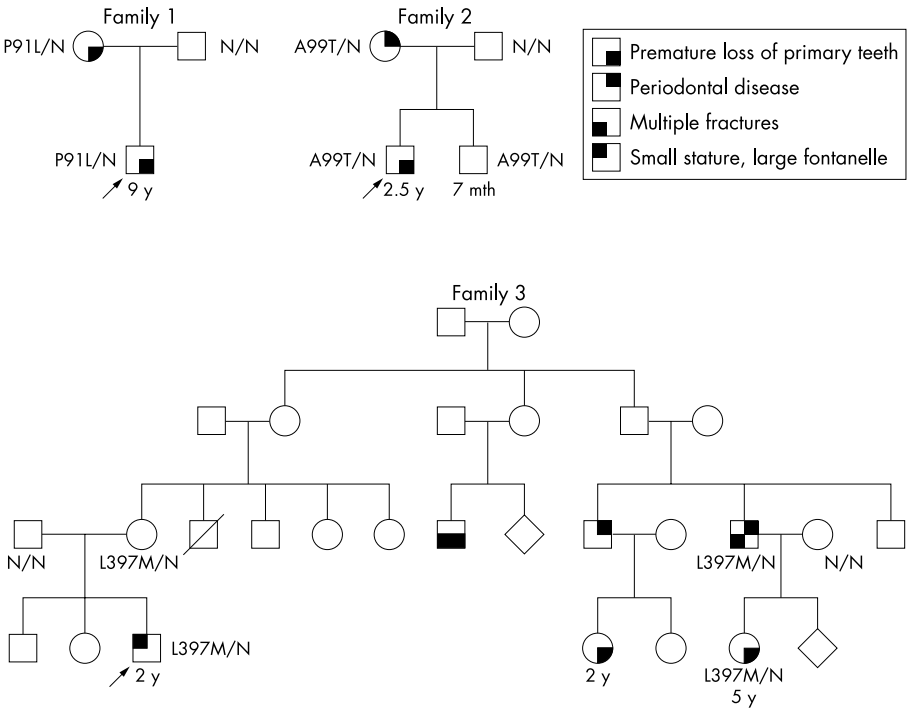

Figure 1 Pedigree data and mutation analysis results in the three families with odontohypophosphatasia. The arrow indicates the proband. N = normal allele as determined by the absence of any detectable mutation.

mostly incisors, began at the age of 2 years. Serum alkaline phosphatase was low (80 U/l, normal range >100). *X* rays showed normal growth plate development and normal long bones without evidence of fractures or rickets. The 39 year old mother of the proband had lost her permanent teeth. Her serum alkaline phosphatase level was low (18 U/l, normal range 30-120). The father did not show any symptoms and had a normal level of serum alkaline phosphatase (93 U/l, normal range 30-120).

Patient 2

The proband was a 2 year old male who has lost three teeth and was referred to the genetics department by his dentist. Serum alkaline phosphatase was low (64 U/l, normal range 100-320) and urinary phosphoethanolamine was high (583 µmol/g creatinine, normal range <350). The parents deny any problem with multiple fractures, bowing of legs, or loss of teeth but the 38 year old mother reported being affected by an unusual number of dental cavities and having had numerous treatments of dental root canals. Her serum ALP was low (29 U/l, normal range 47-137) while serum ALP of the proband's father was normal (66 U/l, 47-137). A second child was born in July 2002. At nearly 7 months of age, this baby boy has not shown any symptoms of hypophosphatasia to bring him to clinical attention.

Patient 3

The proband was a 2 year old boy diagnosed with hypophosphatasaemia. Serum alkaline phosphatase was repeatedly low (84 and 86 U/l, normal range 104-345) and urinary phosphoethanolamine was high (935 µmol/g creatinine, normal range 108-533). He had very slight leg bowing and was just below the 5th centile for height. The mother had a low ALP level (32 U/l, normal range 30-107) and high PEA (138 µmol/g creatinine, normal range 20-100). The father showed normal ALP and PEA levels. The proband's 5 year old maternal second cousin was diagnosed with hypophosphatasia because of early loss of primary teeth and low serum ALP (92 U/l, normal range 108-317). Her skeletal survey and growth/stature were normal for age. Her father had early loss of teeth, multiple caries, and had had four fractures. Another second cousin could not be tested but was reported to have lost her primary teeth at the age of 2.

METHODS

Primer sequences of the 12 *ALPL* gene exons have been previously reported[24] and allowed analysis of the whole coding sequence, including intron-exon borders and untranslated exons. PCR reactions were performed and analysed as previously described.[24] Site directed mutagenesis of the mutation P91L was performed with the Quikchange Site Directed Mutagenesis kit (Stratagene). Mutated and wild type plasmids were transiently transfected in COS-1 cells using the Lipofectamine PLUS reagent (Life Technologies) according to a methodology described previously.[9 26] The mutations were put into a 3D model of the TNSALP molecule[13] by using the molecular visualisation program RasMol (R. Sayle, Glaxo Research and Development, Greenford).

RESULTS

Pedigree data and mutation analysis results are shown in fig 1. In family 1, the proband's mother was affected by early loss of teeth, suggesting that the disease could not be put down to the proband's Down syndrome condition only[35] and that the disease was dominantly inherited.

Sequencing of the *ALPL* gene showed that the patient and her affected mother carried a 323C>T nucleotide substitution resulting in the missense mutation P91L (fig 2). The presence of this mutation in the affected parent and the absence of any other detectable mutation in the patient is consistent with dominant inheritance. The P91L mutation has not previously been reported in hypophosphatasia patients. We therefore introduced it into the expression plasmid pcDNA-3 by site directed mutagenesis and transfected COS-1 cells with the mutant plasmid. We found that the mutation exhibited 0.4% of wild type activity, that is, no or very low residual enzymatic activity, suggesting that this mutation is not a polymorphism and corresponds to a severe allele.[26] In family 2, we found in

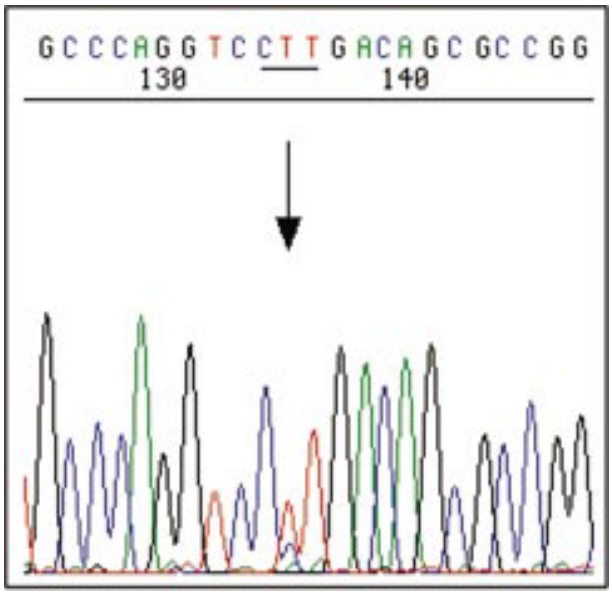

Figure 2 DNA sequencing electropherogram of the proband in family 1 showing the newly identified mutation P91L. The arrow indicates the position of the nucleotide substitution CCT (proline) > CTT (leucine).

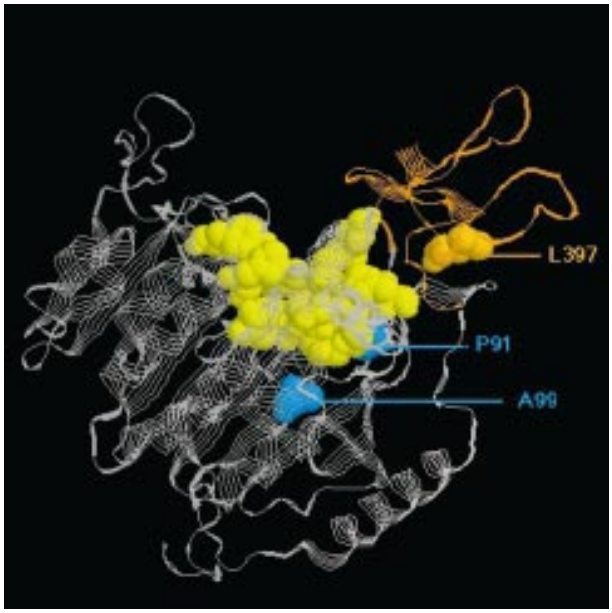

Figure 3 Location of the residues affected by the mutations P91L, A99T, and L397M in the 3D model. The active site is shown in yellow. The crown domain is shown in orange.

the proband the heterozygous nucleotide substitution 346G>A resulting in the missense mutation A99T. The mother reported being affected by dental problems. She showed a low level of serum ALP and carried the mutation A99T, while the father exhibited normal serum ALP and no ALPL gene mutation. The second child, born in July 2002, was prenatally found to be heterozygous for the A99T mutation. Although the mother did not exhibit the typical signs of odontohypophosphatasia (loss of teeth), these results suggest that in this family, heterozygosity for A99T resulted in clinical symptoms but that the disease was minimally expressed in the mother. The mutation A99T was previously described in a large family with dominant odontohypophosphatasia[8] and site directed mutagenesis and transfections of COS cells previously showed that A99T does not allow any significant in vitro residual activity and shows a negative dominant effect.[9] In family 3, the proband did not show any symptoms of hypophosphatasia and was referred to the genetics department because of hypophosphatasaemia. We found the heterozygous substitution 1240C>A resulting in the missense mutation L397M of maternal origin. Interestingly, the proband's second cousin and this cousin's father were affected by odontohypophosphatasia and carried the L397M mutation found in the proband. Exhaustive sequencing of the *ALPL* gene of the affected cousin did not show evidence of any other mutation. This suggests that in this family, heterozygotes for L397M may be affected by the disease and that its expression was subject to intrafamilial heterogeneity. The L397M mutation was previously reported by Mumm *et al*,[32] associated with the D277A mutation, in a patient affected by perinatal hypophosphatasia. This suggests that, like P91L and A99T, L397M is a severe allele. We finally concluded that the disease in these three families resulted from heterozygosity for a severe hypophosphatasia allele.

Localisation of the mutated residues in the 3D model of *TNSALP* based on the placental ALP structure[13] showed that L397M is located in the crown domain, a mammalian specific region observed for the first time in the placental alkaline phosphatase structure and containing a collagen binding loop[13] (fig 3). Alanine 99 is located in an alpha helix running from the active site to the surface of the molecule near the homodimer interface and supporting D92, S93, and A94, three residues of the active site involved in phosphate binding. By disturbing this helix, mutation A99T could therefore affect the active site. Proline 91 is in contact with the active site and there is no doubt that the change of this residue for leucine has an important effect on the catabolic activity. Thus, the study of the 3D model suggests that these mutations alter the function of the enzyme rather than have a structural effect resulting in the degradation of the molecule. This is consistent with the dominant effect of these mutations and the allosteric properties of the enzyme.[36]

DISCUSSION

Considerable variation occurs in the clinical expression of severe forms of hypophosphatasia, owing to the considerable allelic heterogeneity of the *ALP* gene.[33] Moderate forms of hypophosphatasia, especially odontohypophosphatasia, are not as well documented. Compared to bone forms of hypophosphatasia, only a few mutations responsible for odontohypophosphatasia have been published[8 22 28 31] (this study), but they suggest that similar variation exists in these forms, at both the clinical and genetic levels. In family 2, the patient was found to be heterozygous for the A99T mutation, a mutation also found in a large family with dominant hypophosphatasia.[8] The probands from the previously reported family were a 6 year old girl and her fraternal twin brother, both affected with premature loss of anterior teeth at the age of 3.5, and abnormal urinary PEA and serum PLP values. In addition to premature loss of teeth, the probands were affected by very slight bone symptoms, such as thin cortical bone of the cranium and multiple radiolucent spots in the cranial bones, but no additional skeletal abnormalities. In this article, the authors point out the intrafamilial clinical heterogeneity of the disease in carriers of A99T since the clinical signs were evident in eight carriers of the mutation and absent in subjects without the mutation and in five carriers. This intrafamilial heterogeneity was also observed in family 3 where the carriers of L397M showed variable expression of the disease, ranging from only hypophosphatasaemia to periodontal disease associated with multiple fractures. However, the absence of clinical symptoms in the proband could be because of his still young age (2 years), although another second cousin was reported to have lost her primary teeth at the

age of 2 (fig 1). Finally, this report confirms that moderate forms of hypophosphatasia are also highly variable in their clinical expression, owing to allelic heterogeneity but also to other factors that remain to be determined, such as other sequence variations in the ALPL gene, a *trans* effect of other genes, or environmental factors.

Dominant transmission of moderate forms of hypophosphatasia has been documented in a few families.[3 5–9] We report here the case of one additional family with dominant odontohypophosphatasia (family 1) and two others in which heterozygotes for a *TNSALP* gene mutation show clinical symptoms, however variable in expression. In our experience, we failed to detect a second mutated allele in 18% of our hypophosphatasia patients, 70% of them being affected by moderate (childhood, adult, or odonto-) hypophosphatasia (E Mornet, unpublished data). In some of these patients, mutations of the *ALPL* gene may have not been detected because of their location in intronic or regulatory sequences, or because they correspond to large deletions undetectable by the methodology routinely used here. In others, however, the disease may be the result of heterozygosity and no other mutation needed to be sought. Analysis of the transmission of the odontohypophosphatasia phenotype, together with serum ALP level and presence or absence of the mutation, may help to distinguish between the two situations.

The mechanism of dominance remains unclear but probably involves interactions between monomers of the dimeric structure that disturb the allosteric properties of TNSALP. We and others have previously reported that some *ALPL* gene mutations result in a dominant negative effect owing to complete or partial inhibition of the normal monomer by the mutated monomer in the dimeric molecule.[7 9] Here, we show that residues mutated in these families are localised in the vicinity of functional regions such as the active site and the crown domain, suggesting that they may have a functional role. This is consistent with the expected localisation of mutations resulting in an inhibitory effect.

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REFERENCES

1 **Whyte MP**. Hypophosphatasia and the role of alkaline phosphatase in skeletal mineralization. *Endocr Rev* 1994;**15**:439-61.
2 **Brittain JM**, Oldenburg TR, Burkes EJ Jr. Odontohypophosphatasia: report of two cases. *ASDC J Dent Child* 1976;**43**:106-11.
3 **Eberle F**, Hartenfels S, Pralle H, Kabisch A. Adult hypophosphatasia without apparent skeletal disease: "odontohypophosphatasia" in four heterozygote members of a family. *Klin Wochenschr* 1984;**62**:371-6.
4 **Beumer J**, Trowbridge HO, Silverman S Jr, Eisenberg E. Childhood hypophosphatasia and the premature loss of teeth. A clinical and laboratory study of seven cases. *Oral Surg Oral Med Oral Pathol* 1973;**35**:631-40.
5 **Whyte MP**, Fallon MD, Murphy WA. Adult hypophosphatasia: clinical, laboratory and genetic investigation of a large kindred with review of the literature. *Medicine* 1979;**58**:329-47.
6 **Eastman JR**, Bixler D. Clinical, laboratory, and genetic investigations of hypophosphatasia: support for autosomal dominant inheritance with homozygous lethality. *J Craniofac Genet Dev Biol* 1983;**3**:213-34.
7 **Müller HL**, Yamazaki M, Michigami T, Kageyama T, Schönau E, Schneider P, Ozono K. Asp361 mutant of alkaline phosphatase found in patients dominantly inherited hypophosphatasia inhibits the activity of the wild-type enzyme. *J Clin Endocrinol Metab* 2000;**85**:743-47.
8 **Hu JCC**, Plaetke R, Mornet E, Zhang C, Sun X, Thomas HF, Simmer JP. Case report of a family with dominant hypophosphatasia. *Eur J Oral Sci* 2000;**108**:189-94.
9 **Lia-Baldini AS**, Muller F, Taillandier A, Gibrat JF, Mouchard M, Robin B , Simon-Bouy B, Serre JL, Aylsworth AS, Bieth E, Delanote S, Freisinger P, Hu JCC, Krohn HP, Nunes ME, Mornet E. A molecular approach to dominance in hypophosphatasia. *Hum Genet* 2001;**109**:99-108.
10 **Moore CA**, Curry CJR, Henthorn PS, Smith JA, Smith JC, O'Lague P, Coburn SP, Weaver DD, Whyte MP. Mild autosomal dominant hypophosphatasia: in utero presentation in two families. *Am J Med Genet* 1999;**86**:410-15.
11 **Jemmerson R**, Low MG. Phosphatidylinositol anchor of HeLa cell alkaline phosphatase. *Biochemistry* 1987;**26**:5703-9.
12 **Hessle L**, Johnson KA, Anderson HC, Narisawa S, Sali A, Goding JW, Terkeltaub R, Millan JL. Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *Proc Natl Acad Sci U S A* 2002;**99**:9445-9.
13 **Mornet E**, Stura E, Lia-Baldini AS, Stigbrand T, Ménez A, Le Du MH. Structural evidence for a functional role of human tissue non-specific alkaline phosphatase in bone mineralisation. *J Biol Chem* 2001;**276**:31171-8.
14 **Greenberg CR**, Evans JA, McKendry-Smith S, Redekopp S, Haworth JC, Mulivor R, Chordiket BN. Infantile hypophosphatasia localization within chromosome region 1p36.1.1-34 and prenatal diagnosis using linked DNA markers. *Am J Hum Genet* 1990;**46**:286-92.
15 **Weiss MJ**, Ray K, Henthorn PS, Lamb B, Kadesch T, Harris H. Structure of the human liver/bone/kidney alkaline phosphatase gene. *J Biol Chem* 1988;**263**:12002-10.
16 **Weiss MJ**, Cole DEC, Ray K, Whyte MP, Lafferty MA, Mulivor RA, Harris H .A missense mutation in the liver/bone/kidney alkaline phosphatase gene causing a lethal form of hypophosphatasia. *Proc Natl Acad Sci USA* 1988;**85**:7666-9.
17 **Henthorn PS**, Raducha M, Fedde KN, Lafferty MA, Whyte MP. Different missense mutations at the tissue-nonspecific alkaline phosphatase gene locus in autosomal recessively inherited forms of mild and severe hypophosphatasia. *Proc Natl Acad Sci USA* 1992;**89**:9924-8.
18 **Orimo H**, Hayasshi Z, Watanabe A, Hirayama T, Hirayama T, Shimada T. Novel missense and frameshift mutations in the tissue-nonspecific alkaline phosphatase gene in a Japanese patient with hypophosphatasia. *Hum Mol Genet* 1994;**3**:1683-4.
19 **Ozono K**, Yamagata M, Michigami T, Nakajima S, Sakai N, Cai G, Satomura K, Yasui N, Okada S, Nakayama M . Identification of novel missense mutations (Phe310Leu and Gly439Arg) in a neonatal case of hypophosphatasia. *J Clin Endocrinol Metab* 1996;**81**:4458-61.
20 **Orimo H**, Goseki-Sone M, Sato S, Shimada T. Detection of deletion 1154-1156 hypophosphatasia mutation using TNSALP exon amplification. *Genomics* 1997;**42**:364-6.
21 **Fukushi M**, Amizuka N, Hoshi K, Ozawa H, Kumagai H, Omura S, Misumi Y, Ikehara Y, Oda K. Intracellular retention and degradation of tissue-nonspecific alkaline phosphatase with a Gly317Asp substitution associated with lethal hypophosphatasia. *Biochem Biophys Res Commun* 1998;**246**:613-18.
22 **Goseki-Sone M**, Orimo H, Iimura T, Takagi Y, Watanabe H, Taketa K, Sato S, Mayanagi H, Shimada T, Oida S. Hypophosphatasia: identification of five novel missense mutations (G507A, G705A, A748G, T1155C, G1320A) in the tissue-nonspecific alkaline phosphatase gene among Japanese patients. *Hum Mutat Suppl* 1998;**1**:S263-7.
23 **Sugimoto N**, Iwamoto S, Hoshimo Y, Kajii E. A novel missense mutation of the tissue-nonspecific alkaline phosphatase gene detected in a patient with hypophosphatasia. *J Hum Genet* 1998;**43**:160-4.
24 **Mornet E**, Taillandier A, Peyramaure S, Kaper F, Muller F, Brenner R, Bussière P, Freisinger P, Godard J, Le Merrer M, Oury JF, Plauchu H, Puddu R, Rival JM, Superti-Furga A, Touraine RL, Serre JL, Simon-Bouy B. Identification of fifteen novel mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene in European patients with severe hypophosphatasia. *Eur J Hum Genet* 1998;**6**:308-14.
25 **Taillandier A**, Zurutuza L, Muller F, Simon-Bouy B, Serre JL, Bird L, Brenner R, Boute O, Cousin J, Gaillard D, Heidemann PH, Steinmann B, Wallot M, Mornet E. Characterization of eleven novel mutations (M45L, R119H, 544delG, G145 V, H154Y, C184Y, D289 V, 862+5A, 1172delC, R411X, E459 K) in the tissue-nonspecific alkaline phosphatase (TNSALP) gene in patients with severe hypophosphatasia. *Hum Mutat* 1999;**13**:171-2.
26 **Zurutuza L**, Muller F, Gibrat JF, Taillandier A, Simon-Bouy B, Serre JL, Mornet E Correlations of genotype and phenotype in hypophosphatasia. *Hum Mol Genet* 1999;**8**:1039-46.
27 **Mochizuki H**, Saito M, Michigami T, Ohashi H, Koda N, Yamaguchi S. Severe hypercalcaemia and respiratory insufficiency associated with infantile hypophosphatasia caused by two novel mutations of the tissue-nonspecific alkaline phosphatase gene. *Eur J Pediatr* 2000;**159**:375-9.
28 **Taillandier A**, Cozien E, Muller F, Merrien Y, Bonnin E, Fribourg C, Simon-Bouy B, Serre JL, Bieth E, Brenner R, Cordier MP, De Bie S, Fellmann F, Freisinger P, Golembowski S, Hennekam RCM, Josifova D, Kerzin-Storrar L, Leporrier N, Zabot MT, Mornet E. Fifteen new mutations

(-195T, L-12X, 298-2G, T117 N, A159T, R229S, 997+2A, E274X, A331T, H364R, D389G, 1256delC, R433H, N461I, C472S) in the tissue-nonspecific alkaline phosphatase (TNSALP) gene in patients with hypophosphatasia. *Hum Mutat* 2000;**15**:293.
29 **Orimo H**, Girschick HJ, Goseki-Sone M, Ito M, Oda K, Shimada T. Mutational analysis and functional correlation with phenotype in German patients with childhood-type hypophosphatasia. *J Bone Miner Res* 2001;**16**:2313-19.
30 **Watanabe H**, Hashimoto-Uoshima M, Goseki-Sone M, Orimo H, Ishikawa I. A novel point mutation (C571T) in the tissue-non-specific alkaline phosphatase gene in a case of adult-type hypophosphatasia. *Oral Dis* 2001;**7**:331-5.
31 **Taillandier A**, Lia-Baldini AS, Mouchard M, Robin B, Muller F, Simon-Bouy B, Serre JL, Bera-Louville A, Bonduelle M, Eckhardt J, Gaillard D, Myhre AG, Kortge-Jung S, Larget-Piet L, Malou E, Sillence D, Temple IK, Viot G, Mornet E. Twelve novel mutations in the tissue-nonspecific alkaline phosphatase gene (ALPL) in patients with various forms of hypophosphatasia. *Hum Mutat* 2001;**18**:83-4.
32 **Mumm S**, Jones J, Finnegan P, Henthorn PS, Podgornik MN, Whyte MP. Denaturing gradient gel electrophoresis analysis of the tissue nonspecific alkaline phosphatase isoenzyme gene in hypophosphatasia. *Mol Genet Metab* 2002;**75**:143-53.
33 **The Tissue Nonspecific Alkaline Phosphatase Gene mutations database**, http://www.sesep.uvsq.fr/Database.html
34 **Mornet E**. Hypophosphatasia: the mutations of the tissue-nonspecific alkaline phosphatase gene. *Hum Mutat* 2000;**15**:309-15.
35 **Saxen L**, Aula S. Periodontal bone loss in patients with Down's syndrome: a follow-up study. *J Periodontol* 1982;**53**:158-62.
36 **Hoylaerts MF**, Manes T, Millan JL. Mammalian alkaline phosphatases are allosteric enzymes. *J Biol Chem* 1997;**272**:22781-7.

RPGR mutation associated with retinitis pigmentosa, impaired hearing, and sinorespiratory infections

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Retinitis pigmentosa (RP) is a progressive retinal degeneration that affects about 1 in 4000 of the population.[1] Approximately 15-30% of patients with RP have X linked retinitis pigmentosa (XLRP), which is the most severe form of RP consistently manifesting early in life.[2 3] Night blindness is usually present in early childhood with loss of peripheral visual fields and ultimately central vision, resulting in registered blindness by the end of the third decade. Female carriers display a broad spectrum of fundus appearances ranging from normal to extensive retinal degeneration.[4–6]

XLRP is genetically heterogeneous with two major loci, RP2 (Xp11.23) and RP3 (Xp21.1). Both disease genes have now been identified (respectively *RP2*[7] and *RPGR*[8–10]) with *RP2* mutations causing disease in approximately 15% of XLRP families,[11 12] while *RPGR* mutations are reportedly more common, accounting for up to 75% of XLRP.[10] Two other rare loci for XLRP have also been described on Xp22 and Xq26-27.[13 14]

Hong *et al*[15] described the phenotype and pathology of an *RPGR* knockout mouse model. They showed the subcellular localisation of RPGR to the photoreceptor connecting cilia, and in the absence of RPGR partial mislocalisation of essential outer segment proteins. These data suggest a putative role for RPGR in the retina, controlling movement of essential proteins from the inner to the outer segment of photoreceptors via the connecting cilia. Several groups have recently identified a retina specific RPGR interacting protein (RPGRIP1).[16–18] This protein also localises to the photoreceptor connecting cilium and is thought to be a structural component of the ciliary axoneme.[18] Subsequent mutation screening in patients suffering from retinal diseases has identified mutations in *RPGRIP1* as a cause of Leber congenital amaurosis.[19 20]

In this report, we present the phenotype of a family suffering from XLRP associated with hearing loss, sinusitis, and chronic recurrent respiratory tract infections. To identify the causative gene on the X chromosome, we performed haplotype analysis with subsequent mutation screening of candidate genes. The new phenotype described is associated with a mutation in the *RPGR* gene, and highlights the significance of RPGR protein kinociliary function in non-ocular tissue.

Key points

- We report a novel systemic phenotype associated with XLRP, with patients suffering from hearing loss, sinusitis, and chronic chest infections, suggesting a mutation in a gene involved in ciliary function.
- The phenotype overlaps those described for primary ciliary dyskinesia and Usher syndrome.
- Genetic analysis of this family has identified a frameshift mutation in exon 8 of the *RPGR* gene.
- A gene in close proximity to *RPGR*, *TCTEL1*, was also examined for cSNPs as a potential phenotypic modifier locus; none was detected.
- Our findings show that mutations in the *RPGR* gene are associated with a complex phenotype broadening the clinical spectrum of disease, and provide supporting evidence for an essential ciliary function for RPGR in the retina and other tissues.
- RPGR and interacting partners involved in kinociliary function in a variety of tissues may therefore represent attractive candidate genes for other diseases, such as primary ciliary dyskinesia or hearing loss.

PATIENTS AND METHODS

Patients and controls

Appropriate informed consent was obtained from the family and control volunteers under investigation. An X linked form of retinitis pigmentosa was established by pedigree analysis, clinical examination, and ophthalmological tests. Blood samples were collected from each available member of the family and from controls and DNA extracted using the Nucleon II Kit (Scotlab Limited) according to the manufacturer's instructions. Clinical characterisation included ophthalmic and systemic history, visual field testing, and fundus examination. In addition fundus photography was performed. Three

The first two authors contributed equally to this work